STIMULATION BY COENZYME Q_{10} OF THE GROWTH OF A MYCOBACTERIUM*

James O. Norman and Robert P. Williams

Department of Microbiology
Baylor University College of Medicine
Houston 25, Texas

Received May 3, 1960

Coenzyme Q compounds have been shown to possess coenzymatic properties in mitochondrial electron transport systems (Green and Lester, 1959). In particular the coenzyme activity is involved in the succinoxidase and succinic-cytochrome c reductase activity of particulate material obtained from animal tissues. Coenzyme Q also has been obtained from certain bacteria (Lester and Crane, 1959), but interestingly enough not from the family of mycobacteria. The latter organisms are rich in vitamin K compounds, and Brodie and Ballantine (1960) have established that vitamin K_1 or a closely related homologue is involved in oxidative phosphorylation by extracts of Mycobacterium phlei. No reports have appeared describing growth stimulatory properties to coenzyme Q compounds. The present communication describes experiments indicating that coenzyme Q_{10} possesses growth stimulatory activity for a species of Mycobacterium.

The strain of mycobacterium employed in this investigation was isolated from a human case of pulmonary disease. The organism is an atypical nonphotochromogenic mycobacterium labeled Strain III which has the characteristics of the scotochromogenic variety of mycobacteria as described by Timpe and Runyon (1954). Inocula were harvested from Lowenstein-Jensen egg medium (BBL),

*This investigation was supported by research grant E-2365 from the Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

treated for a few minutes in a tissue homogenizer to break up clumps, washed, and diluted in the medium of Proskauer and Beck (1898) so that the suspension permitted only 90 per cent light transmission when measured in a Coleman Junior spectrophotometer at 660 mμ. The inoculum consisted of 0.2 ml of this suspension in 10 ml of test medium.

Growth experiments were carried out in the liquid medium of Proskauer and Beck (1898). This medium contains asparagine as a nitrogen source and glycerol as an energy source in addition to the usual inorganic salts. Strain III will grow in the medium after 2-3 wk without the addition of any other substances. Cultures were incubated without shaking in screw capped test tubes at 37°C. Growth response was determined turbidimetrically by measuring optical density in a Coleman Junior spectrophotometer.

Crystalline coenzyme Q_{10} was the gift of Dr. Karl Folkers of Merck, Sharp and Dohme Research Laboratories. Hydroquinone, quinone (1, 4 benzoquinone), menadione, and vitamin K_1, the latter two as preparations suitable for intravenous use, were commercial preparations. Vitamin K_1 was added directly to the medium; the other compounds were dissolved in ethyl ether. After addition of the solutions to the medium, excess ether was driven off by heating the tubes to 56°C for one hour. Casein hydrolyzate was Sheffield Farms "N-Z Case", and the yeast extract was Difco. Sodium lauryl sulfate was prepared in distilled water, and sterilized by filtration.

Figure 1 shows the response of the test organisms to various concentrations of coenzyme Q_{10}, as well as the effect of sodium lauryl sulfate. It is evident that the detergent, sodium lauryl sulfate, is required before a growth response with coenzyme Q_{10} can be obtained. In experiments not illustrated here it was demonstrated that the concentration of sodium lauryl sulfate influenced the growth response to coenzyme Q_{10}. At concentrations below 15 μg per ml the growth of the organism in 10 μg of coenzyme Q_{10} per ml was not above control cultures containing no coenzyme. Sodium lauryl sulfate by itself did not stimulate growth. Sodium choleate in concentrations similar to sodium lauryl sulfate also was effective in bringing about a growth response to

coenzyme Q_{10}. In addition to being required for growth stimulation, sodium lauryl sulfate also permitted dispersed growth of the mycobacteria in the presence of coenzyme Q_{10}.

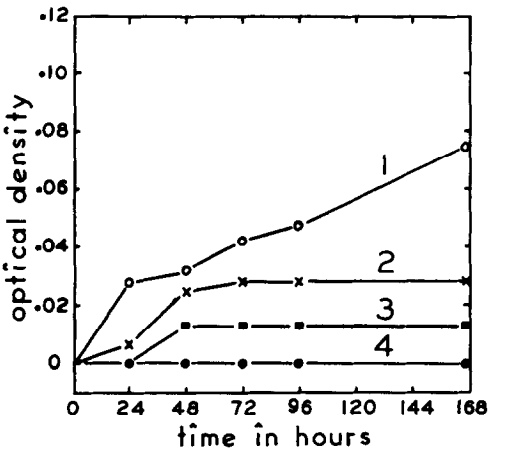

Figure 1. Effect of coenzyme Q_{10} and sodium lauryl sulfate upon growth of nonphotochromogenic mycobacterium Strain 111. 1) 100 ug Q_{10} per ml with 25 ug per ml sodium lauryl sulfate; 2) 10 ug Q_{10} per ml with sodium lauryl sulfate; 3) 1 ug Q_{10} per ml with sodium lauryl sulfate; 4) no Q_{10} or 10 ug Q_{10} without sodium lauryl sulfate.

As can be seen in figure 1, the growth response of the organism was greater with increasing concentrations of coenzyme Q_{10}. The difference in response is particularly noted in the first 24 hr of incubation. The fact that after 72 hr growth leveled off in 1 and 10 ug concentrations suggests that the amount of coenzyme present in medium limits the total growth of the organism. Similar experiments carried out with Mycobacterium tuberculosis H37Ra and H37Rv demonstrated that coenzyme Q_{10} and sodium lauryl sulfate did not stimulate the growth of these organisms.

Several other compounds in addition to coenzyme Q_{10} have been tested to determine if they will stimulate growth of the organism. These compounds are listed in table 1. The only substance giving a similar response was vitamin K_1. Menadione (vitamin K) was inactive, as were quinone and hydroquinone which have ring structures similar to coenzyme Q_{10}. Vitamin K_1 is a naphthoquinone compound whereas coenzyme Q_{10} is a quinone. However, both are charac-

terized by having isoprenoid units in the side chain. Subcultures of organisms from coenzyme Q_{10} and vitamin K_1 cultures established that the organisms were viable, and that growth could be maintained upon continued subculture.

Although coenzyme Q compounds have been found to be active in restoring enzymatic function to succinoxidase and succinic-cytochrome c reductase systems, this is the first report describing growth stimulatory activity for one of the compounds. The coenzyme Q substances isolated from bacterial sources have been characterized by the lower homologues than Q_{10} (Lester and Crane, 1959).

TABLE 1

Growth Response of Nonphotochromogenic Mycobacterium to Coenzyme Q_{10} and Other Substances

Substance	Activity	Substance	Activity
Coenzyme Q_{10}*	+	Quinone*	-
Menadione*	-	Tween 80	-
Vitamin K_1*	+	Casein hydrolyzate	-
Hydroquinone*	-	Yeast extract	-

Activity means an increase in optical density at 168 hr of test cultures over control cultures.

*These compounds were dissolved in ethyl ether and added to the media in concentrations of 10 µg per ml. Sodium lauryl sulfate (25 µg per ml) also was included in these cultures.

Green and Lester (1959) have reported that detergent agents greatly enhance the interaction of coenzyme Q_{10} with mitochondrial particles. However, the lower homologues (coenzyme Q_2) restore enzymatic activity without the addition of detergent. Presumably the detergent facilitates the entry of the coenzyme into the particle. A similar situation might explain the requirement of a detergent for growth stimulation by coenzyme Q_{10}. Lower homologues of the coenzyme, such as exist naturally in bacteria, might not require the addition of detergent.

Stimulation of bacterial growth by vitamin K compounds has been reported (Lev, 1959). The organism described, Fusiformis nigrescens, also required meat extract, blood, and anaerobic conditions before growth occurred. Of greater interest are the experiments of Brodie and coworkers. They (Brodie Davis, and Fieser, 1958) have isolated a naphthoquinone compound similar to vitamin K_1 from M. phlei. The compound, as well as vitamin K_1, specifically restored oxidation and phosphorylation to light-treated bacterial extracts (Brodie and Ballantine, 1960). Oxidation alone was restored by certain benzoquinone compounds, including coenzyme Q_{10}, but to a very limited extent. Brodie, Russell, and Kashket (1960) have suggested that benzoquinones might play a role in the energy metabolism of microorganisms. Such a possibility could explain the role of coenzyme Q_{10} in stimulating the growth of scotochromogenic mycobacterium Strain 111.

REFERENCES

Brodie, A. F. and Ballantine, J. J. Biol. Chem. 235, 232 (1960).

Brodie, A. F., Davis, B. R., and Fieser, L. F. J. Am. Chem. Soc. 80, 6454 (1958).

Brodie, A. F., Russell, P. J., and Kashket, E. Abstracts of Papers, Am. Chem. Soc., 137th Meeting, 25C (1960).

Green, D. E. and Lester, R. L. Fed. Proc. 18, 987 (1959).

Lester, R. L. and Crane, F. L. J. Biol. Chem. 234, 2169 (1959).

Lev, M. J. Gen. Microbiol. 20, 697 (1959).

Proskauer, B. and Beck, M. Ztschr. Hyg. Infectionskrankh. 18, 128 (1898).

Timpe, A. and Runyon, E. H. J. Lab. Clin. Med. 44, 202 (1954).